DETAILED ACTION

1. Applicant's response filed on July 19, 2011 is acknowledged. Claims 1-23 are pending. Claim 1 has been amended. Claims 17-23 have previously been withdrawn. Claims 1-16 are currently under examination.

Information Disclosure Statement

2. The information disclosure statement (IDS) submitted on July 19, 2011 is in compliance with the provisions of 37 CFR 1.97. Accordingly, the information disclosure statement is being considered by the examiner.

Objections Withdrawn

3. In view of Applicant's amendment, the objection to claim 1 because on first sight the acronym "OMV" should be followed by "Outer Membrane Vesicle" is withdrawn.

Rejections Withdrawn

4. In view of Applicant's arguments, the rejection of claims 1-4 and 10-13 under 35 U.S.C. 102(b) as being anticipated by Fukasawa et al. (Vaccine, 1999; 17: 2951-2958) is withdrawn.

5. In view of Applicant's arguments, the rejection of claims 1-7 and 10-15 under 35 U.S.C. 103(a) as being unpatentable over Fukasawa et al. (Vaccine, 1999; 17: 2951-

2958), and further in view of Berthet et al. (US 2006/0204520 A1; Filed: 2/8/02) is withdrawn.

Rejections Maintained

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

6. The rejection of claims 1- 5, 8-13 and 16 under 35 U.S.C. 103(a) as being unpatentable over Granoff et al. (U.S. Patent 6,936,261 B2; Filed 7/27/01) and further in view of van Reis et al. (Current Opinion in Biotechnology, 2001; 12: 208-211) is maintained for the reasons set forth in the previous office action.

Applicant argues that:

1) Granoff et al. do not expressly teach the claimed "step of ultrafiltration of a crude OMV preparation containing bacterial DNA."

2) Similarly, van Reis et al. do not expressly teach that ultrafiltration may be performed in the presence of contaminating "bacterial DNA".

3) Nowhere in Granoff et al. do they teach that a crude OMV preparation containing bacterial DNA is subjected to ultrafiltration. There is no mention as to how to deal with bacterial DNA and the reference only references filtration, not ultrafiltration.

4) van Reis et al. fail to remedy the deficiencies of Granoff et al. because van Reis et al. do not teach that HPTFF can be performed on crude OMV preparations without first removing the bacterial DNA.

5) The advantages cited by the Examiner asserting that one of skill in the art would use HPTFF as taught by van Reis et al. are all directed to the utility of HPTFF in purifying proteins.

6) Unless the Examiner can provide evidence or sound reasoning that one of skill in the art would follow the teachings of Granoff et al. and necessarily perform HTPFF of a crude OMV preparation containing bacterial DNA, the Examiner has not established that Granoff et al. when combined with van Reis et al. inherently renders the claimed invention obvious.

Applicant's arguments have been considered, but are deemed non-persuasive

Independent claim 1 is drawn to a process for preparing bacterial Outer Membrane Vesicles (OMVs) comprising a step of ultrafiltration of a crude OMV preparation containing bacterial DNA prior to any ultracentrifugation or sterilization steps.

Independent claim 16 is drawn to a process for purifying bacterial OMVs, wherein the process does not a centrifugation step in which the OMVs are pelleted.

With regard to Points 1-3 and 6, in response to applicant's arguments against the references individually, one cannot show nonobviousness by attacking references individually where the rejections are based on combinations of references. See *In re Keller*, 642 F.2d 413, 208 USPQ 871 (CCPA 1981); *In re Merck & Co.*, 800 F.2d 1091,

231 USPQ 375 (Fed. Cir. 1986). The claims are rejected under 35 U.S.C. 103(a), which means that Granoff et al., on its own, would not recite each limitation of the claimed invention. If that were true the rejection would have been an anticipatory reference for which it is not. Granoff et al. do not specifically disclose the use of ultra-filtration and thus van Reis et al. was incorporated to provide the teachings lacking in Granoff et al. Van Reis et al. provides the teaching of ultra-filtration in combination with a proper motivation to use ultra-filtration in the method of Granoff et al. The combination of references meets the limitations of the instantly claimed invention because ultrafiltration membranes and process designs can provide the high concentration factors and greater overall yield required for high-dose products (see page 210; conclusions).

Moreover, with regard to having a preparation containing bacterial DNA, the Examiner is of the position that the OMV preparation of Granoff et al. necessarily contains bacterial DNA because DNA is packaged within the membrane derived vesicles of gram negative bacteria; as evidenced by Dorward et al., Applied and Environmental Microbiology, 1990; 56(6): 1960-62. Consequently, since Granoff et al. did not do anything to remove DNA from the OMV preparation, absence any evidence to the contrary, said preparation contains DNA.

Lastly, in response to applicant's argument that the references fail to show certain features of applicant's invention, it is noted that the features upon which applicant relies (i.e., performed in the presence of contaminating bacterial DNA) are not recited in the rejected claim(s). Although the claims are interpreted in light of the

specification, limitations from the specification are not read into the claims. See *In re Van Geuns*, 988 F.2d 1181, 26 USPQ2d 1057 (Fed. Cir. 1993).

With regard to Point 4, van Reis et al. remedies the deficiencies of Granoff et al. for the reasons set forth in the previous office action (provided below for convenience). The claims do not require that ultrafiltration be performed on crude OMV preparations without first removing the bacterial DNA.

With regard to Point 5, while the advantages cited by the Examiner asserting that one of skill in the art would use the HPTFF as taught by van Reis et al. are directed towards purifying proteins, one of ordinary skill in the art recognizes that using the techniques of HPTFF are not limited to proteins and recognizes that said filtration technique is a rapid and efficient method for filtration and separation of solutions containing biomolecules in general or particles such as viruses, bacteria or cellular material, as evidenced by the teachings of Tangential Flow Filtration; http://www.pall.com/main/Biopharmaceuticals/Tangential-Flow-Filtration-Introduction-51907.page).

As previously presented, Granoff et al. disclose compositions comprising OMV against diseases caused by *Neisseria meningitidis*, specifically serogroup B strains (see title and abstract). Granoff et al. disclose a composition comprising outer membrane vesicles (OMV) prepared from the outer membrane of a cultured strain of Neisseria meningitidis spp. OMVs may be obtained from a Neisseria meningitidis grown in broth or solid medium culture, preferably by separating the bacterial cells from the culture medium by filtration, lysing the cells (e.g. by addition of detergent, osmotic shock, sonication, cavitation, homogenization, or the like) and separating an outer membrane fraction from cytoplasmic molecules by filtration; outer membrane fractions may be used to produce OMVs (see column 14, lines 19-35). Granoff et al. disclose

that their experiments included outer membrane vesicle vaccine prepared from *N. meningitidis* serogroup B strain, H44/76 (B:15:P1.7,16; "Norwegian vaccine"), (see column 4, lines 7-14). Moreover, Granoff et al. disclose that the composition may include an adjuvant (see column 15, lines 18-22).

Granoff et al. do not specifically disclose the use of ultra-filtration as recited in claim 1; that the ultrafiltration is cross flow or tangential flow as recited in claim 4; or that the membrane used for ultrafiltration has a cut off of about 300kDA as recited in claim 5.

van Reis et al. disclose membrane separations in biotechnology, which include microfiltration and ultrafiltration for protein concentration and buffer exchange. van Reis et al. disclose that ultrafiltration (UF) has become the method of choice for protein concentration and buffer exchange, largely replacing size-exclusion chromatography. UF devices have been developed in which the pressure, fluid flow, and concentration profiles remain constant when changing the scale of operation. Equal flow distribution among channels/fibers is achieved using appropriate piping manifolds and proper design of entrance and exit regions. The system allows 1000-fold volumetric scaling of UF process with consistent protein yield and process flux. Moreover, a new control strategy in which concentration of retained protein at the membrane surface remains constant has been developed, which enables processes with enhanced product yield, minimal membrane area, consistent process time and greater robustness with respect to variations in feed quality and membrane properties (see page 209; Ultrafiltration). Tangential flow microfiltration using 0.2 µm-rated membranes generates a particle free harvest solution that requires no additional clarification before subsequent purification. The systems are operated at a constant flux instead of constant trans-membrane pressure to improve product yield and throughput (see page 208). van Reis et al. disclose that high performance tangential flow filtration (HPTFF) is an emerging technology for protein purification. It is a two-dimensional unit operation that exploits both size and charge mechanisms. Additionally, protein concentration, purification and buffer exchange can be accomplished in a single unit operation. HPTFF has equivalent throughput to UF (see page 209; High-performance tangential flow

filtration). The ultrafiltration step of van Reis et al. absent evidence to the contrary, necessarily results in diafiltration.

With regard to claim 16, which requires that the process not include a centrifugation step in which the OMVs are pelleted, the process of Granoff discloses that separating an outer membrane fraction from cytoplasmic molecules can be done by filtration; *or* by differential precipitation *or* aggregation of outer membranes and/*or* outer membrane vesicles, *or* by affinity separation methods using ligands that specifically recognize outer membrane molecules; *or* by a high-speed centrifugation that pellets outer membranes and/*or* outer membrane vesicles, or the like (see column 14, lines 27-35), which the Examiner is interpreting "or" as in an alternative option to the others listed. As such, the process of Granoff to exclude a centrifugation step in which the OMVs are pelleted is disclosed by Granoff.

Limitations such as the cut off weight of the ultrafiltration membrane are being viewed as limitations of optimizing experimental parameters.

It would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to modify the invention of Granoff et al. with the ultrafiltration of van Reis et al. to prepare bacterial OMVs, comprising a step of ultrafiltration prior to any ultracentrifugation or sterilization steps because ultrafiltration membranes and process designs can provide the high concentration factors and greater overall yield required for high-dose products (see page 210; conclusions). Moreover, it would have been prima facie obvious to use ultrafiltration that is tangential flow because high performance tangential flow filtration (HPTFF) is a two-dimensional unit operation that exploits both size and charge mechanisms, is an emerging technology for protein purification, has equivalent throughput to ultrafiltration and is sufficient to accomplish protein concentration, purification and buffer exchange in a single unit operation.

One would have had a reasonable expectation, barring evidence to the contrary, that the method would be effective for a process for preparing bacterial OMVs.

Since the claimed method steps were known in the prior art and one skilled in the art could have combined the steps as claimed by known methods with no change in

their respective functions and the combination would have yielded predictable results to one of ordinary skill in the art at the time of the invention.

7. The rejection of claims 6, 7, 14 and 15 under 35 U.S.C. 103(a) as being unpatentable over Granoff et al. (U.S. Patent 6,936,261 B2; Filed 7/27/01) and further in view of van Reis et al. (Current Opinion in Biotechnology, 2001; 12: 208-211) as applied to claims 1-5, 8-13 and 16 above, and further in view of Berthet et al. (US 2006/0204520 A1; Filed: 2/8/02) is maintained for the reasons set forth in the previous office action.

Applicant argues that:

1) Granoff et al. when combine with van Reis et al. do not expressly teach or suggest all of the claimed elements and do not inherently teach or suggest all of the claimed elements. Therefore Granoff et al. when combined with van Reis et al. do not render the present claims obvious. The Examiner has not established that Berthet et al. remedy this deficiency.

Applicant's arguments have been considered, but are deemed non-persuasive.

Independent claim 1 is drawn to a process for preparing bacterial OMVs comprising a step of ultrafiltration of a crude OMV preparation containing bacterial DNA prior to any ultracentrifugation or sterilization steps.

Dependent claim 6 is drawn to the process of claim 1, wherein the OMVs are sterilized after ultrafiltration.

Dependent claim 7 is drawn to the process of claim, wherein the sterilization is by filter sterilization.

Dependent claim 14 is drawn to the process of claim 1, wherein the *N. meningitidis* has one or more mutations to decrease or knockout expression of a gene product.

Dependent claim 15 is drawn to the process of claim 14, wherein the gene product is Cps, CtrA, CtrB, CtrC, CtrD, ExbB, ExbD, FrpB, GalE, HtrB, MsbB, LbpA, LbpB, LpxK, NMB0033, OpA, OpC, PhoP, PilC, PmrE, PmrF, PorA, PorB, rmpM, SiaA, SiaB, SiaC, SiaD, SynA, SynB, SynC, TbpA and/or TbpB.

With regard to Point 1, as set forth supra and contrary to Applicant's assertion, the combination of Granoff et al. in view of van Reis et al. expressly teaches and suggests all the claimed elements. The claims are rejected under 35 U.S.C. 103(a), which means that Granoff et al., on its own, would not recite each limitation of the claimed invention. If that were true the rejection would have been an anticipatory reference for which it is not. Granoff et al. do not specifically disclose the use of ultra-filtration and thus van Reis et al. was incorporated to provide the teachings lacking in Granoff et al. Van Reis et al. providing the teaching of ultra-filtration in combination with a proper motivation to use ultra-filtration in the method of Granoff et al. The combination of references meets the limitations of the instantly claimed invention because ultrafiltration membranes and process designs can provide the high concentration factors and greater overall yield required for high-dose products (see page 210; conclusions).

Moreover, with regard to having a preparation containing bacterial DNA, the Examiner is of the position that the OMV preparation of Granoff et al. necessarily

contains bacterial DNA because DNA is packaged within the membrane derived vesicles of gram negative bacteria; as evidenced by Dorward et al., Applied and Environmental Microbiology, 1990; 56(6): 1960-62. Consequently, since Granoff et al. did not do anything to remove DNA from the OMV preparation, absence any evidence to the contrary, said preparation contains DNA. Moreover, the combination of Granoff et al., van Reis et al. and Berthet et al. renders the present claims obvious.

As previously presented, the teachings of Granoff et al. have been described previously. The teaching of van Reis et al. have been described previously.

Granoff et al. and van Reis et al. do not specifically disclose that the OMVs are sterilized after ultrafiltration as recited in claim 6; that the sterilization is by filter sterilization as recited in claim 7; that the *N. meningitidis* has one or more mutations to decrease or knockout expression of a gene product as recited in claim 14; or that the gene product is Cps, CtrA, CtrB, CtrC, CtrD, ExbB, ExbD, FrpB, GalE, HtrB, MsbB, LbpA, LbpB, LpxK, NMB0033, OpA, OpC, PhoP, PilC, PmrE, PmrF, PorA, PorB, rmpM, SiaA, SiaB, SiaC, SiaD, SynA, SynB, SynC, TbpA and/or TbpB as recited in claim 15.

Berthet et al. disclose a process for preparing bacterial OMV's of engineered Gram-negative bacterial strains that have improved outer-membrane vesicle shedding properties (see paragraph 0002). Berthet et al. disclose that vesicles prepared from such modified strains may have reduced particle size, which allows sterile filtration through 0.22 µm pores (see paragraph 0058). Moreover, Berthet et al. disclose that many bacterial outer membrane components are present such as PorA, PorB, Opc, Opa, FrpB and possess observed protection; others such as TbpB have been defined as being relevant to the induction of protective immunity (see paragraph 0013). Berthet et al. disclose that preferred Neisserial (particularly, Neisseria *meningitidis* B) vesicle preparations include one or more preferred genes selected from PorA, PorB, PilC, ThpA, TbpB, LbpA, LbpB, Opa, and Opc or htrB, MsbB and lpxK for down regulation (see paragraphs 0091-93).

It would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to modify the invention of Granoff et al. and van Reis et al. as combined above, with the teachings of Berthet et al. to modify said strain with one or more mutation to decrease or knockout expression of a gene product because purification of blebs is technically difficult; bleb production in most Gram-negative strains results in poor yields of product for the industrial production of vaccines. Therefore, modifying said strain solves this problem and provides specially modified "hyperblebbing" strains from which vesicles may be more easily made in higher yield and may be more homogeneous in nature. Said vesicles may then be readily filter sterilized. Additionally, if the bacteria produce more vesicles then the usual process steps to remove detergent such as purification and centrifugation may be obviated (see Berthet et al.; paragraphs 0036-0037). Moreover, it would be prima facie obvious to one of ordinary skill in the art at the time the invention was made to modify the invention of Granoff et al. and van Reis et al. with the teachings of Berthet et al. to modify the specific genes as claimed because they are the most common genes of many bacterial outer membrane components.

Lastly, it would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to modify the invention of Granoff et al. and van Reis et al. with the teachings of Berthet et al. to sterilize the OMVs by filter sterilization because it utilizes a filter with smaller pores which will limit the amount of material that may become clogged along the process.

One would have had a reasonable expectation, barring evidence to the contrary, that the method would be effective for a process for preparing bacterial OMVs.

Since the claimed method steps were known in the prior art and one skilled in the art could have combined the steps as claimed by known methods with no change in their respective functions and the combination would have yielded predictable results to one of ordinary skill in the art at the time of the invention. See the recent Board decision *Ex parte Smith,--USPQ2d--*, slip op. at 20, (Bd. Pat. App. & Interf. June 25, 2007) (citing *KSR, 82 USPQ2d* at 1396).

Conclusion

8. No claim is allowed.

9. **THIS ACTION IS MADE FINAL.** Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the mailing date of this final action.

10. Any inquiry concerning this communication or earlier communications from the examiner should be directed to LaKia Tongue whose telephone number is (571)272-2921. The examiner can normally be reached on Monday-Friday 8-5:30.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Gary Nickol can be reached on 571-272-0835. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/LaKia Tongue/
Examiner, Art Unit 1645
October 7, 2011

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